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EFFECTS OF SEX HORMONES ON MOUSE MAMMARY CANCER CELLS IN VITRO

by

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I. INTRODUCTION

It has been suggested by many animal experiments and clinical experiences that there is a certain relation between sex hormones and the development of tumors. SCHINZINGER (1889) first pointed out the relation between mammary carcinoma and sex hormones, and BEATSON (1896) obtained a satisfactory result by treatment of inoperable cases of mammary carcinoma with oophorectomy. LATHROP & LOEB (1916) reported that the ovary played some part in the incidence of mammary carcinoma in animal experiments. LACASSAGNE (1932) succeeded in producing mammary carcinoma in 3 male mice by the successive injections of an estrogenic hormone. It has been shown that follicle hormone is an important factor in the incidence of mammary carcinoma, along with genetic and milk factors. On the other hand, according to LACASSAGNE & RAYNAUD (1939), NATHANSON & ANDERVONT (1939), JONES (1941), HEIMAN (1944) and others, male hormones inhibit or decrease the incidence of mammary carcinoma.

However, the inhibitory effect of male hormones on the development of mammary carcinoma in animal is not satisfactory, as reported by NATHANSON & ANDERVONT (1939) and JONES (1941). Unsatisfactory results also occur in human mammary carcinoma. According to ADAIR & HERMAN (1946), however, male hormone treatment is not effective when given in small doses, and large doses give fairly good effects. Several reports have been presented, so far, that the administration of large doses of male hormone has produced some improvement in signs and symptoms of mammary carcinoma or prolongation of the survival period.

The mode of action of sex hormones on mammary carcinoma has not been well known. Various theories concerning the mechanism have been introduced, such as the direct action of hormones on mammary carcinoma, their indirect action through the mid-brain pituitary gland system, or their effect on protein and electrolyte metabolism.

The author tried to elucidate the mechanism of the action of sex hormones by means of tissue culture of mammary carcinoma.

II. MATERIALS AND METHODS

A) MATERIALS

1) Chicken plasma: Fifteen ml. of blood, anti-coagulated with 1 ml. of 1:2500 heparin, was taken from the wing vein of the chicken which had been kept fasting for 24 hours before bleeding. The plasma was separated from the chicken blood by centrifugation of 2000 r. p. m. for 15 minutes. The withdrawal of blood was performed on the day of experiment, as a rule. The plasma was stored in a refrigerator at $0^{\circ}\sim 5^{\circ}$ C with tight stopper, if it was to be used within a few days.

2) Salt solution: HANKS' balanced salt solution was used. The hydrogen ion concentration was adjusted with isotonic 1.4 per cent solution of NaHCO_3 .

3) Chick embryo extract: Eggs fertilized for 8~9 days were used. After the sterilization of the shell, the broad end of the egg was broken, and the chick embryo was taken out through a hole made on the membranous sac. After a proper number of embryos were collected, they were washed with HANKS' solution and compressed with tissue compressor. The gruel-like juice obtained by this way was centrifugated for 30 minutes by 3000 r. p. m., and the supernatant fluid was used for experiments. This extract was made on the day of experiment because its growth-promoting effect gradually diminishes with time.

4) Horse serum: The serum from the healthy horse was filtrated with SEIZ's apparatus. Then, it was warmed at 56° C for 30 minutes in order to denature specific proteins such as complement. The serum was stored in refrigerator at $0^{\circ}\sim 5^{\circ}$ C.

5) Tissues: Mammary carcinomas developed spontaneously in the mice of NA2 strain were cultured. For the control, the hearts of chick embryos were cultured.

6) Substances tested: Hormones tested were Testosterone propionate, Estradiol, Estrone, Estriol, anterior pituitary hormone and Progesterone. Except for anterior pituitary hormone, all the hormones were suspended in a proper solvent (made by TEIKOKU ZOKI Pharmaceutical Co.) in crystalline form, since they are not watersoluble. The action of Nitromin, one of the anti-carcinomatous agents, was also tested. Anterior pituitary hormone and Nitromin were solved in HANKS' solution.

B) METHODS OF TISSUE CULTURE

The tissue for culture was excised aseptically and washed several times with HANKS' solution. The tissue was then placed on a depressed slide and cut into small pieces of 1 mm^3 in size with a cataract knife. The fragments of tissue were floated in HANKS' solution in the hollow on the slide.

1) Cover-slip method: A drop of chicken plasma was deposited in the center of a coverglass and spread in a circle about 10 mm in diameter. Three pieces of the above-mentioned tissue fragments were picked up and put into the plasma with a metal spatula, and a drop of HANKS' solution containing 20 per cent chick embryo extract and 20 per cent horse serum was added to it. The plasma coagulated like jelly soon after the addition of the drop. A depressed slide was inverted over the coverglass. The cultures were then sealed with melted paraffin and transferred to the incubator. Sometimes the double cover-slip method, introduced by MAXIMOW (1925), was used. For the observation with phase-contrast microscope, the tissue

was cultured on the inner surface of two coverglasses placed on both surfaces of a perforated glass slide.

These methods are convenient for histological examinations of the cultures and for subcultures, but not for prolonged culture.

2) **CARREL flask method:** The **CARREL** flasks of 30 mm in diameter were used. First, 0.15 ml. of chicken plasma was introduced into the flask and agitated slightly to spread the plasma diffusely on the floor of the flask. Five tissue fragments were placed on it at proper distances. Then, 0.40 ml. of **HANKS'** solution containing 10 per cent chick embryo extract was added to it. After coagulation took place, 0.75 ml. of the fluid medium which consisted of chick embryo extract, 20 per cent; horse serum, 20 per cent; and **HANKS'** solution (60 per cent) was added. The neck of the flask was sterilized with a flame and closed with a rubber stopper.

As compared with the cover-slip method, the **CARREL** flask method provides ample nutriment and oxygen to the cultures, and the exchange of the fluid medium or gas is possible. Therefore, it is suitable for prolonged tissue culture and its histological observation.

C) METHODS OF OBSERVATION

1) **Macroscopic examination:** When the culture grew vigorously, the outgrowth was observed around the explant as a whitish semitransparent zone which enlarged with the elapse of days. The metabolism of the cultures changed the pH of the medium to the acid side. This change in pH was indicated by the addition of phenol-red to the medium.

2) **Microscopic examination:** One advantage of tissue culture method is that it permits the direct microscopic observation of living cells growing at body temperature. The movement, division, and internal structure of living and growing cells could be studied with the phase-contrast microscope. The successive morphological changes of living cells were studied with time-lapse cinematography (one frame per 1~20 seconds).

3) **Staining of cultures:** The cultures were fixed with 2 per cent formalin-**RINGER'S** solution for several hours, washed with distilled water and then stained with hematoxylin-eosin.

4) **Measurement of outgrowth:** Numerous methods have been devised for measuring the rate of growth of cells in tissue cultures. The author used the method to measure the increase in the area occupied by tissue fragments from day to day. The **CARREL** flask was placed on the square stand of **EDINGER'S** vertical projectoscope. The illuminated outlines of tissue fragments were traced on paper and their areas were measured with a planimeter. On each occasion that measurements were made, growth indices were calculated as follows:

$$\text{Relative increase} = \frac{\text{total area of culture} - \text{area of original explant}}{\text{area of original explant}}$$

$$\text{Growth index} = \frac{\text{relative increase of experimental fragment}}{\text{relative increase of control fragment}}$$

III. RESULT

A) CULTIVATION OF MAMMARY CARCINOMA

1) Interrelation between histological pictures of mammary tumors and their cultures: It has generally been believed that the cultivation of epithelial cells in vitro is more difficult than that of connective tissue cells. Mammary carcinoma with abundant stroma content is of very hard consistency and difficult to cut sharply. In the cultivation of such tissue, proliferation of fibroblasts frequently takes place. The relation between the histological pictures of mammary tumors and their cultures are as follows:

Various classification of mammary tumors in mice have been reported by different authors. In this study, the author classified mammary tumors following AKAMATSU's classification (1956), into the ductal type, acinar type, metaplastic type and sarcoma. The acinar type, which shows acinar structure and contains a minimal amount of stroma, was the easiest to culture; pure culture in the form of epithelial sheet was possible (Figs. 1~4). Compared with this type, the tumors of the ductal and

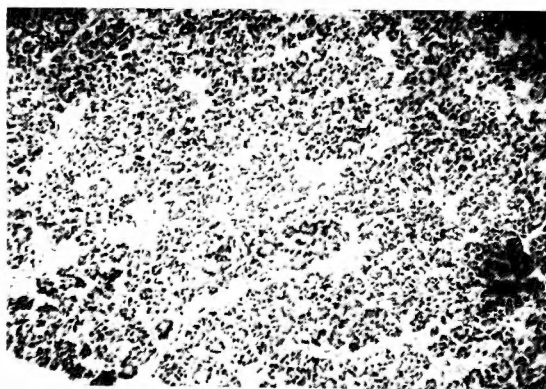


Fig. 1 Spontaneous NA2 mouse mammary carcinoma. Acinar type. $\times 100$

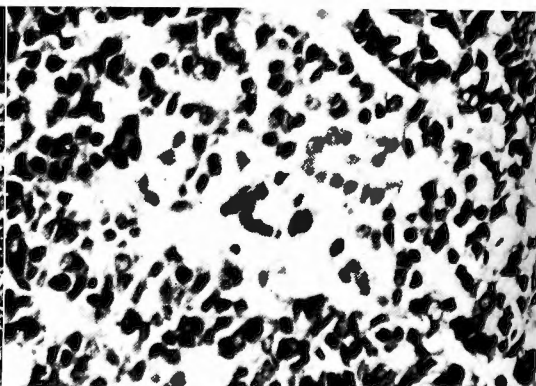


Fig. 2 Higher magnification of the same preparation. $\times 400$

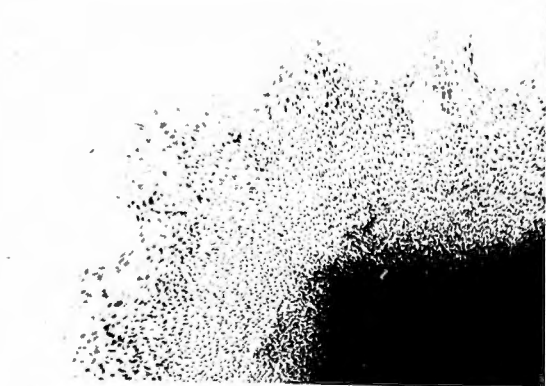


Fig. 3 Culture of mouse mammary carcinoma. The growth forms a sheet of closely adjoining polygonal cells. $\times 50$

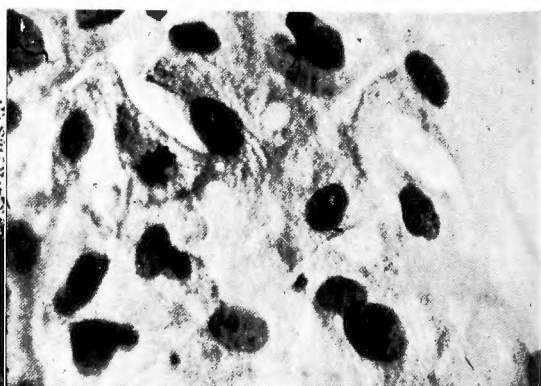


Fig. 4 Higher magnification of the same preparation showing a mitosis in the epithelial sheet. $\times 400$

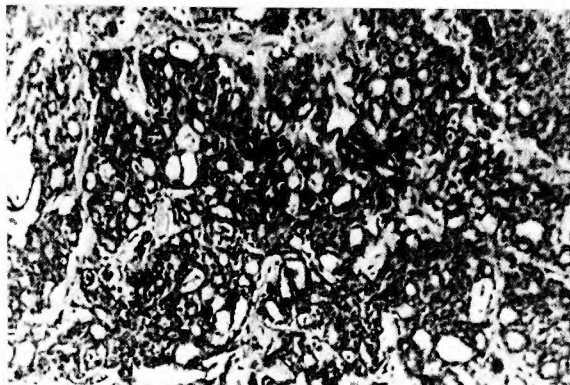


Fig. 5 Ductal type. $\times 100$

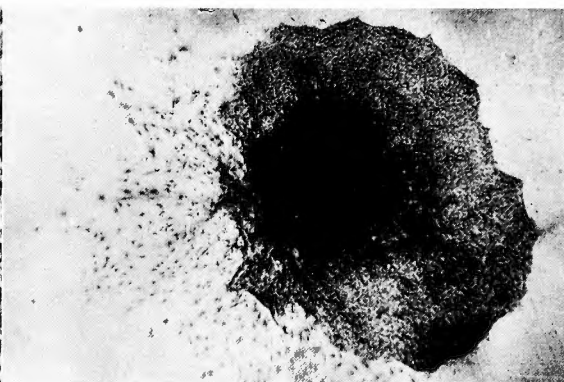


Fig. 6 Culture of mouse mammary carcinoma. Mixed outgrowth of epithelium and fibroblasts. A sheet growth on one side of a culture and spindle cell growth on the other side. $\times 30$

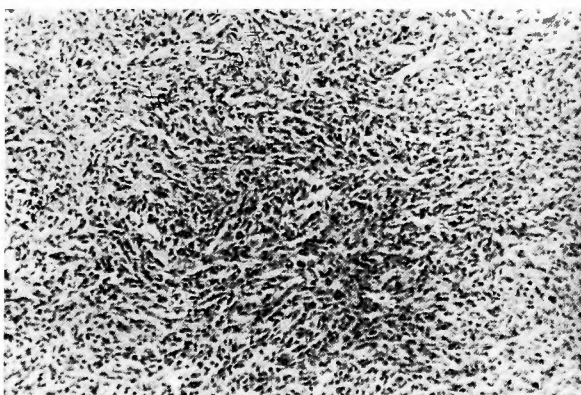


Fig. 7 Sarcoma (fibroblastic, non-epithelial tumor). $\times 100$



Fig. 8 Culture of sarcoma. Fibroblastic outgrowth and the high content of cells of the monocyte-macrophage type. $\times 50$

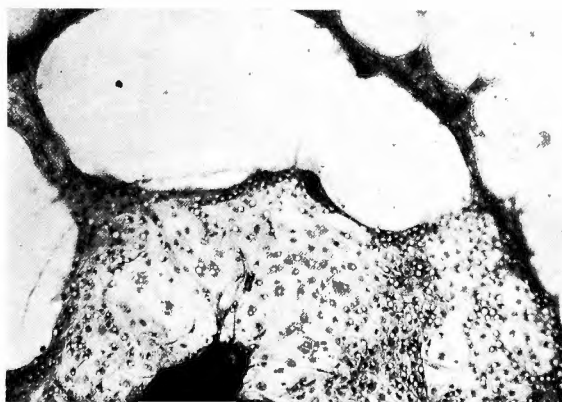


Fig. 9 Note areas of liquefaction. $\times 100$

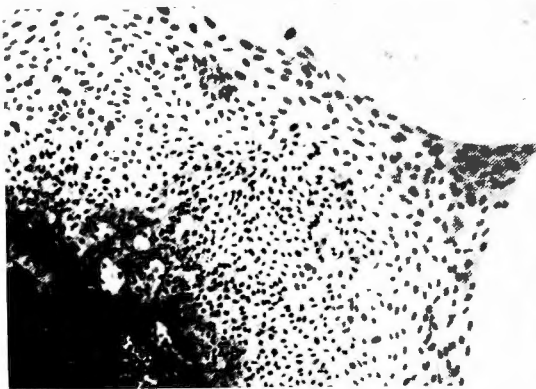


Fig. 10 Culture of mouse mammary carcinoma. Epithelial sheet showing pavement arrangement of one layer. $\times 150$

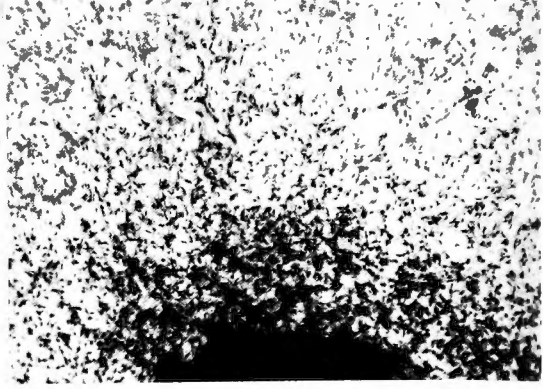


Fig. 11 Culture of chick embryo heart showing a network of fibroblasts. $\times 100$

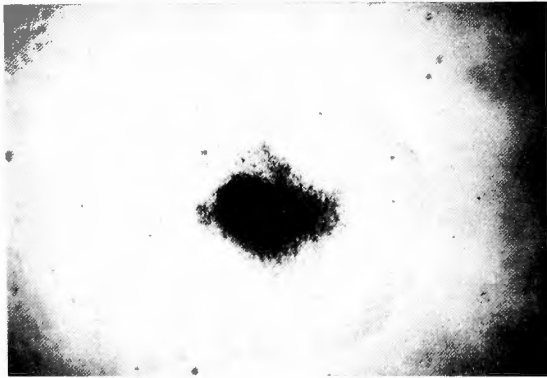


Fig. 12 Living culture of mouse mammary carcinoma. $\times 20$



Fig. 13 Dark-medium phase image $\times 800$

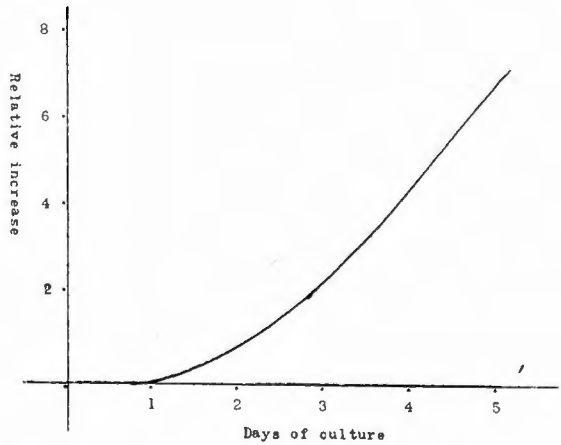


Fig. 14 Growth of mouse mammary carcinoma.



Fig. 15 Structural formation similar to acini. $\times 100$

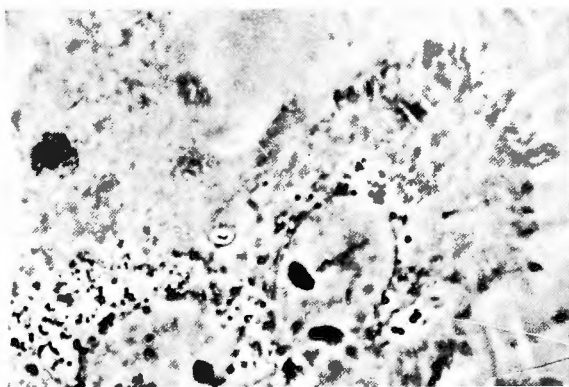


Fig. 16 Living cells. Dark-medium phase image.
 $\times 1800$

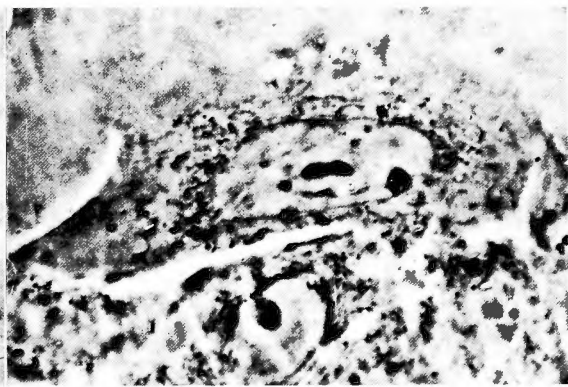


Fig. 17 Living cells. Dark-medium phase image.
 $\times 1800$

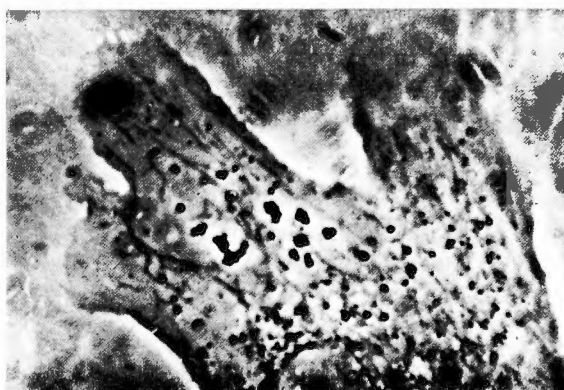


Fig. 18 Note the long filamentous mitochondria. Dark-medium phase image. $\times 1800$

metaplastic types were rich in stroma, and difficult to cut sharply. In the cultivations of these tumors, the mixed outgrowths of epithelium and fibroblasts were observed (Figs. 5 and 6). One of the 53 cases of spontaneous NA2 mouse mammary tumor was a non-epithelial sarcoma. And a peculiar growth, which was different from the ordinary epithelial outgrowth, was seen in its culture. This was characterized by fibroblastic outgrowth and a high percentage of cells of the monocyte-macrophagic type, and was very similar to the culture of chick embryo spleen (Figs. 7 and 8). Thus, the tumors of the acinar type which was the most suitable to pure culture were chosen for the experiments.

2) Culture media: The contents of media used for the cultivation of the mammary carcinoma were described before. The media containing mouse spleen extract instead of chick embryo extract did not favor the growth of this epithelium. Cultivation on the media which consisted of HANKS' solution containing only chick embryo extract was possible, but liquefaction of the plasma and degeneration of the cultivated cells frequently occurred (Fig. 9). The addition of horse serum prevented these phenomena to some extent. The reaction of media was adjusted to pH 8.0 with 1.4 per cent isotonic NaHCO_3 .

3) Growth pattern: Migration of the epithelial cells occurred, centrifugally around the explant at about 24 hours after the cultivation of the mammary carcinoma. The cells were in close continuity with each other and formed an epithelial sheet showing pavement arrangement of one layer (Fig. 10). It was quite different from the net-work of fibroblasts in the culture of chick embryo heart (Fig. 11). Under the ordinary microscope, the borders between the cells were not discernible. Therefore, the cultures were observed as a broad syncytium with scattered nuclei (Fig. 12). Under the phase-contrast microscope, the cultures consisted of closely-adjoining cubic individual cells, some of which in the most peripheral region showing a pseudopodial movement (Fig. 13). Cell divisions were also observed here and there in the sheets (Fig. 4). The increase in the area occupied by tissue fragments was mainly due to the cell division and the migration of the cells. On the 5th day of culture, the area of tissue fragment attained about eight times the size of the original explant (Fig. 14). Very rarely culture showed a structural formation similar to the vesicles or "acini" of the whole organ (Fig. 15).

4) Morphology of the cells: The living cells were observed with the phase-contrast microscope. The cells were cuboid and closely contiguous. The nucleus had a clear margin, with homogenous karyoplasm without any structure. One or two nucleoli were present in a nucleus (Figs. 16 and 17). The mitochondria were slender and elongated as filaments and found scattered in the protoplasm (Fig. 18). Recording by cinematography, these mitochondria showed intricate creeping-worm movements showing splitting, fusing or ramification (Fig. 19).

5) Observations in prolonged culture: When the tissue is cultured with a definite amount of nutriment and oxygen, a decrease in these metabolic requisites and an increase in the harmful excretes occur. And the pH of culture media changes toward the acidic side, so that the living condition of the tissue becomes

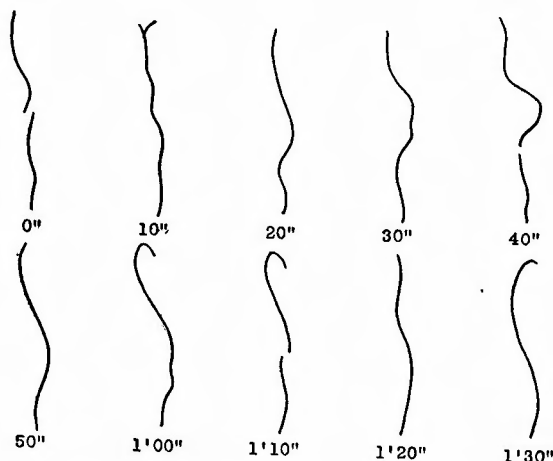


Fig. 19 Movement of mitochondria

Mitochondria fuse or split in cultured cells, and they always undergo changes in position and number.

unsatisfactory with the elapse of days. Then, the tissue ceases developing, and degeneration of the cells occurs. The CARREL flask method allows cultivation for 5~7 days. The cultivation longer than that requires exchange of the fluid media or subculture of the tissue. The author continued the cultivation of mouse mammary carcinoma for about 3 months by the subculture method. By the cover-slip method, the best condition for observation is within 48~72 hours after the explantation of tissue. Degeneration or appearance of vacuoles occurs after that unless subculture is performed.

6) Retransplantations of cultures to mice: The author has not succeeded in retransplantation of the cultures of spontaneous NA2 mouse mammary carcinoma to the same strain of mice. Neither can mammary carcinoma in a NA2 mouse be transplanted to other mice of the same strain. Therefore, the culture is considered to have the same properties as its mother explant. On the other hand, the author succeeded in retransplantation of the cultures of spontaneous C3H mouse mammary carcinoma to the same strain of mice. It was a relatively slow-growing tumor, with a latent period of about 10 days. In general, genetic factors play an important role in the transplantation of mammary carcinoma.

B) THE ACTION OF SEX HORMONES

Various concentrations of hormones have been employed. In each concentration, ten tissue fragments of the mammary carcinoma (in 2 CARREL flasks) were cultured, and the relative increases were obtained by the above mentioned method. For a control, a solvent which did not contain hormone was added to the fluid medium. Each hormone was tried with three different mammary carcinomas. Then the average growth indices were calculated. The same experiments were carried out in the cultures of the chick embryo heart.

1) Testosterone propionate: Testosterone propionate was added to the fluid medium to make the concentration 10^{-1} mg, 10^{-2} mg..... 10^{-5} mg per 1 ml. of medium.

The growth indices were calculated and tabulated in Table 1. Testosterone propionate in high concentrations had an inhibitory action on the growth of both mouse mammary cancer tissue and chick embryo heart tissue, while that in low concentrations was stimulatory to both. The inhibitory action of high concentrations of Testosterone propionate was more marked on the mammary cancer tissue than on the embryo heart tissue.

Table 1 Effect of testosterone propionate

Concentration mg/ml.	Heart (chick embryo)				Mammary cancer (mouse)			
	Days of culture							
	2	3	4	5	2	3	4	5
10 ⁻¹	0.90	0.72	0.76	0.67	0.73	0.65	0.62	0.59
10 ⁻²	1.00	1.08	1.09	1.10	0.91	0.90	0.93	0.87
10 ⁻³	1.10	1.20	1.21	1.32	1.00	1.00	1.03	1.05
10 ⁻⁴	1.00	1.02	1.04	1.05	1.00	1.03	1.14	1.11
10 ⁻⁵	1.00	1.00	1.00	1.02	1.00	1.05	1.17	1.13

2) Estradiol: The same was investigated with Estradiol. The inhibitory action of high concentrations and the stimulatory effect of low concentrations of Estradiol were observed in both tissues. The stimulatory action was more remarkable in the mammary cancer tissue than in the embryo heart tissue (Tab. 2).

Table 2 Effect of estradiol

Concentration ng/ml.	Heart (chick embryo)				Mammary cancer (mouse)			
	Days of culture							
	2	3	4	5	2	3	4	5
10 ⁻¹	0.91	0.90	0.80	0.75	0.92	0.95	0.94	0.95
10 ⁻²	1.09	1.13	1.26	1.27	1.00	1.09	1.26	1.32
10 ⁻³	1.05	1.05	1.14	1.16	1.00	1.09	1.16	1.17
10 ⁻⁴	1.00	1.04	1.07	1.13	1.00	1.05	1.13	1.15
10 ⁻⁵	1.00	1.03	1.02	1.00	1.00	1.05	1.03	1.02

3) Mixture of male and female hormones: Estradiol plus a small dose of Testosterone propionate showed more stimulatory action on the growth of the mammary cancer tissue than Estradiol alone (Tab. 3).

Table 3 Effects of mixed hormones on mouse mammary cancer

Concentration mg/ml.	Days of culture			
	2	3	4	5
Estradiol 10^{-2}	1.00	1.05	1.09	1.20
Estradiol $10^{-2} \times 2/3$ Test. prop. $10^{-2} \times 1/3$	1.00	1.05	1.13	1.31
Estradiol $10^{-2} \times 1/3$ Test. prop. $10^{-2} \times 2/3$	0.89	0.91	0.97	0.96
Test. prop. 10^{-2}	0.89	0.91	0.97	0.93

4) Estrone and Estriol: Estrone had slightly more stimulatory action on the growth of the mammary cancer tissue than Estriol (Tab. 4).

Table 4 Effects of estrone and estriol on mouse mammary cancer

Concentration mg/ml.	Days of culture			
	2	3	4	5
Estrone 10^{-1}	0.91	0.95	0.94	0.88
" 10^{-2}	1.00	1.03	1.09	1.16
" 10^{-3}	1.00	1.00	1.03	1.04
Estriol 10^{-1}	0.90	0.93	0.94	0.86
" 10^{-2}	1.00	1.02	1.06	1.10
" 10^{-3}	1.00	1.00	1.06	1.06

5) Anterior pituitary hormone: Prae-hormon (made by SHIONOGI Pharmaceutical Co.) was employed. The hormone stimulated the growth of both tissues, but the action on the embryo heart tissue was more marked (Tab. 5).

Table 5 Effect of prae-hormon

Concentration unit/ml.	Heart (chick embryo)				Mammary cancer (mouse)			
	Days of culture							
	2	3	4	5	2	3	4	5
10	0.95	1.02	1.02	1.03	0.85	0.90	0.90	0.97
1	1.05	1.19	1.25	1.25	1.02	1.01	1.02	1.05
0.1	1.03	1.05	1.15	1.19	1.05	1.09	1.13	1.18
0.01	1.00	1.00	1.02	1.03	1.00	1.02	1.05	1.02

6) Progesterone: Progesterone stimulated the growth of the mammary cancer tissue slightly more than that of the embryo heart tissue (Tab. 6).

Table 6 Effect of progesterone

Concentration mg/ml.	Heart (chick embryo)				Mammary cancer (mouse)			
	Days of culture							
	2	3	4	5	2	3	4	5
10^{-1}	0.80	0.80	0.78	0.75	0.85	0.84	0.83	0.80
10^{-2}	1.03	1.05	1.10	1.10	1.02	1.05	1.17	1.18
10^{-3}	1.00	1.02	1.02	1.03	1.00	1.00	1.05	1.09
10^{-4}	1.00	1.00	1.01	1.00	1.00	1.00	1.00	1.02

7) Nitromin: The inhibitory action of Nitromin on the tissue growth was more intense than the hormones, and 10^{-1} mg per ml. of the agent completely suppressed the growth of both tissues. With 10^{-2} mg per ml., the tissues again started growing on the 3rd day following the initial inhibition (Tab. 7).

In order to investigate the histological changes in the cultures, these were fixed and stained with hematoxylin-eosin on the 4th day after being cultured in CARREL' flasks on the media to which Testosterone propionate or Estradiol was added. In the cultures on the media with high concentrations of the hormones, the shrunken

Table 7 Effect of nitromin

Concentration mg/ml.	Heart (chick embryo)				Mammary cancer (mouse)			
	Days of culture							
	2	3	4	5	2	3	4	5
10^{-1}	0	0	0	0	0	0	0	0
10^{-2}	0	0.20	0.38	0.46	0	0.15	0.36	0.45
10^{-3}	0.69	0.92	0.94	0.95	0.62	0.80	0.89	0.95
10^{-4}	0.90	1.00	1.00	1.00	0.75	0.95	1.00	1.00

cytoplasm and pyknotic nuclei were observed. These degenerative changes were more intensively observed in the cultures on the media with high concentrations of Testosterone propionate (Figs. 20, 21 and 22). When low concentrations of these hormones were applied, the cultures were in good condition, and no degenerative changes were observed (Figs. 23 and 24). Nitromin in concentration of 10^{-1} mg per ml. of fluid medium, when added to the 3rd day cultures, caused a remarkable cellular degeneration and the cells fell off the glass wall of the flask (Figs. 25 and 26).

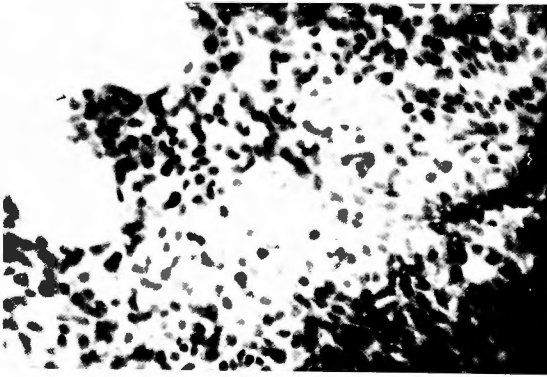


Fig. 20 Control mouse mammary cancer cells. $\times 200$



Fig. 21 Mouse mammary cancer cells treated with 0.1 mg of testosterone propionate/ml. of medium for 3 days. $\times 200$

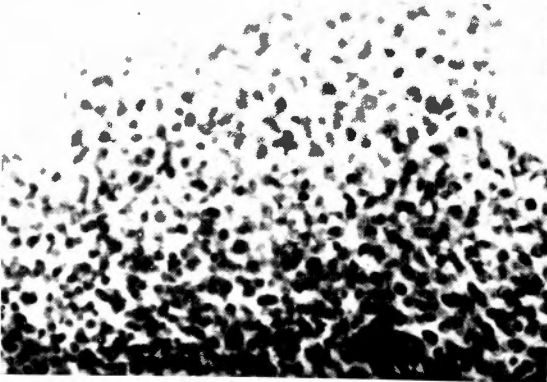


Fig. 22 Mouse mammary cancer cells treated with 0.1 mg of estradiol/ml. of medium for 3 days. $\times 200$

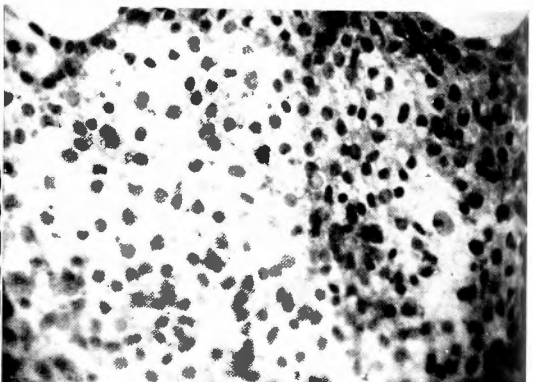


Fig. 23 Mouse mammary cancer cells treated with 10^{-5} mg of testosterone propionate/ml. of medium for 3 days. $\times 200$

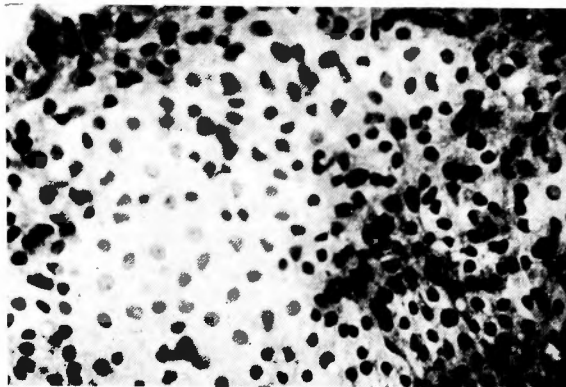


Fig. 24 Mouse mammary cancer cells treated with 10^{-2} mg of estradiol/ml. of medium for 3 days. $\times 200$

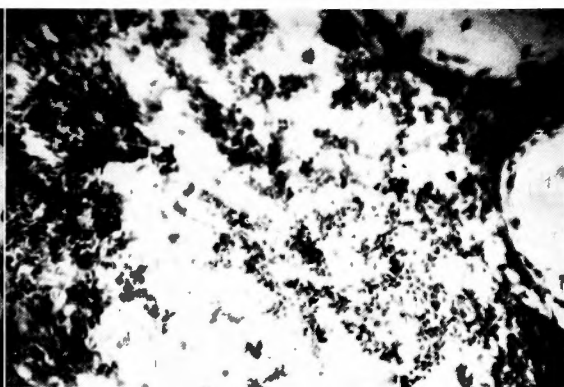


Fig. 25 Mouse mammary cancer cells treated with 0.1 mg of nitromin/ml. of medium. $\times 100$

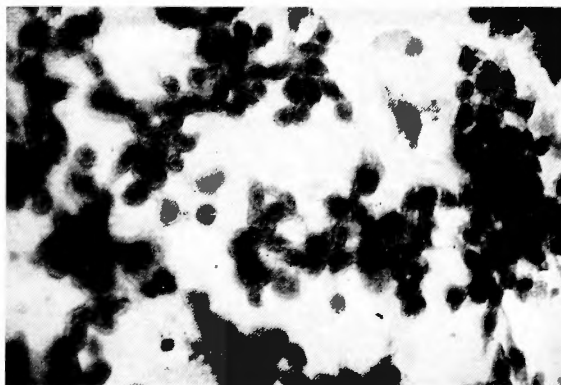


Fig. 26 Higher magnification of the same preparation. $\times 400$

Cinematography revealed that the movements of mitochondria became suppressed and the protoplasmic granules gathered around the nuclei with the addition of the growth-inhibitory concentrations of hormones to the cultures.

IV. DISCUSSION

In the cultivation of mammary carcinoma, it has been reported that epithelial cells and fibroblasts usually grow together, since the tissue contains a large amount of stroma. LUDFORD (1932) stated that a good sheet growth on the cultivation of mammary gland had rarely been obtained except with explants of glands taken at the early stage of pregnancy. LASFARGUES (1957) recommended the application of collagenase to the normal mammary tissue before its culture, since the main factors opposing the epithelial out-growth are the fatty tissues embedding so richly the

tubular network of the gland and the dense collagen frame-work of the main part of the ground substance of this tissue. The author used the acinar type of mammary carcinoma, which contained a minimal amount of stroma as compared with the other types of mammary carcinoma, for cultivation with satisfactory results.

PIKOVSKI (1954) reported that he succeeded in pure culture of mouse mammary carcinoma by keeping pH of culture media alkaline (9~9.3) in order to prevent proliferation of fibroblasts. In my experience, alkaline media certainly prevented the proliferation of fibroblasts, but, at the same time, suppressed the epithelial growth to some extent. Therefore, pH of the media was kept at 8.

There have been reports that cultures of glandular tissue also show organization, such as formation of tubules and vesicles. EBELING & FISHER (1922), DREW (1923), and CHLOPIN (1932) stated that fibroblasts were necessary for the differentiation of epithelial cells. In the author's experiments, the differentiation of cultures was very rare. In these cases, it seemed that the fibroblasts mixed with epithelial cells caused the differentiation. LASFARGUES (1957) reported that, in the cultivation of the normal mammary gland, the fibroblasts and epithelial cells developed in close symbiosis, without competing with each other, and that the fibroblasts seemed to induce an early organization of the epithelium.

According to PIKOVSKI (1954), cultures of mammary carcinoma of RIII strain mouse could be retransplanted to the direct descendant of the donor of the carcinoma, but not to another subgroup of the same strain. The author has not succeeded in retransplantation of the cultures of spontaneous NA2 mouse mammary carcinoma to the same strain of mice. But the same experiments were performed in C3H strain mice and all resulted successfully. These suggest that the genetic factor plays an important role in the retransplantation of the cultures of mouse mammary carcinoma to the mice.

The studies on the influences of sex hormones on the cultures have been performed by ZAKROZEWSKI (1929), OMMYOJI (1932), YAGI (1937) and others, and their growth-stimulating effect in low concentrations has been reported. MÖLLENDORF (1941), noticing the relation between sex hormones and the development of tumor, reported that sex hormones applied to the cultures of fibroblasts did not suppress the growth rate but caused abnormal mitosis. Very few reports on the application of sex hormones to the cultures of mammary carcinoma could be found. LASFARGUES (1957) observed that the addition of placental serum to the cultures of normal mouse mammary gland yielded satisfactory growth, and suspected that the steroid hormones which were contained in the placental serum in a high concentration caused the favorable response of the epithelium. LASNITZKI (1954) reported that Estrone applied to the prostate glands taken from older mice in vitro caused a considerable stimulation of the fibromuscular stroma with atrophy of the alveolar epithelium. IMAGAWA & SYVERTONE (1954) reported that the mouse mammary cancer cells in culture were severely damaged by exposure to samples of guinea pig anti-serum representative of tissues known to contain the mammary tumor virus.

The author observed that, in vitro, sex hormones did not have specific action on

mammary carcinoma, but there were some differences in their action on the mouse mammary carcinoma and on the chick embryo heart. This experiment suggests that a large dosage of hormones should be used in treatment of mammary carcinoma, but that dramatic results from hormone therapy for this disease have not yet been demonstrated. In evaluating the author's study, the following points must be taken into consideration.

- 1) When the study is performed *in vivo*, the administration of a sex hormone causes change in hormonal balance of the body and changes the effect of the sex hormone.

- 2) Higher concentration of a substance is necessary for the same physiological action to take place *in vitro* than *in vivo*.

- 3) The hormone-effect in mammary carcinoma depends on the strain of mouse, malignancy of mammary carcinoma, and the degree of its differentiation. The author cultured the acinar type of mammary carcinomas developed in NA2 strain mice, which were considered to be differentiated to a high degree.

V. SUMMARY AND CONCLUSION

In order to study the direct action of sex hormones on mammary carcinoma, the mammary carcinomas developed spontaneously in the NA2 strain mice were cultured *in vitro* and sex hormones were added to them. For a control, the same experiments were performed with chick embryo hearts.

The following results were obtained.

- 1) In general, sex hormones in high concentrations inhibited and these in low concentrations stimulated the growth of the mammary cancer tissue of mouse.

- 2) The results in comparison with the tissue of chick embryo heart were as follows:

- i) Testosterone propionate in high concentrations inhibited the growth of the mammary cancer tissue more markedly than that of the chick embryo heart tissue.

- ii) Estradiol stimulated the growth of the mammary cancer tissue more markedly than that of the chick embryo heart tissue.

- iii) Anterior pituitary hormone stimulated the growth of the chick embryo heart tissue more than that of the mammary cancer tissue.

- iv) Progesterone stimulated the growth of the mammary cancer tissue slightly more than that of the chick embryo heart tissue.

- 3) A mixture of male and female hormones. Estradiol plus a small dose of Testosterone propionate showed more stimulatory action on the growth of the mammary cancer tissue than Estradiol alone.

- 4) Estrone had more stimulatory action on the growth of the mammary cancer tissue than Estradiol.

From this study, it has been concluded that sex hormones have certain direct effects on mouse mammary cancer cells *in vitro*.

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和 文 抄 録

体外培養マウス乳癌に対する性ホルモンの作用

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マウス乳癌組織は培養24時間前後から次第に発育を認め、その発育状態は線維芽細胞の網状の発育とは全く相異なる上皮細胞性の膜状の増殖であつた。ヘマトキシリン・エオジン染色で細胞は鋪石様の配列を示し、各所にミトーゼを認めた。

乳癌培養組織に対する性ホルモンの直接作用は次の様であつた。

1. 一般的にいつて、種々の性ホルモンはその高濃度に於て発育抑制的に、低濃度では発育促進的に作用した。

2. 併し特に乳癌培養組織に対する性ホルモンの作用を鶏胎児心臓培養組織に対する場合と比べると

i) Test·prop. は高濃度に於て、乳癌組織に対する発育抑制作用が鶏胎児心臓組織に対するよりも強かつた。

ii) Estradiol の場合は乳癌組織に対しての方が

より発育促進的であつた。

iii) 脳下垂体前葉ホルモンの場合は、両者に対して発育促進的に作用するが、鶏胎児心臓組織に対してはより促進的であつた。

iv) Progesterone の場合は乳癌組織に対しての方が少々発育促進的に作用した。

3. 男女性混合ホルモン。乳癌組織に Estradiol を単独に作用させた場合よりも、これに Test·prop. を僅かに加えた場合の方がより発育促進的であつた。

4. Estrone 及び Estriol. 乳癌組織に対して Estrone の方が Estriol より 少々発育促進的に作用した。

以上の in vitro 実験成績から、性ホルモンは乳癌細胞の発育に直接或程度の影響を与えるものではないだろうかと推論するものである。